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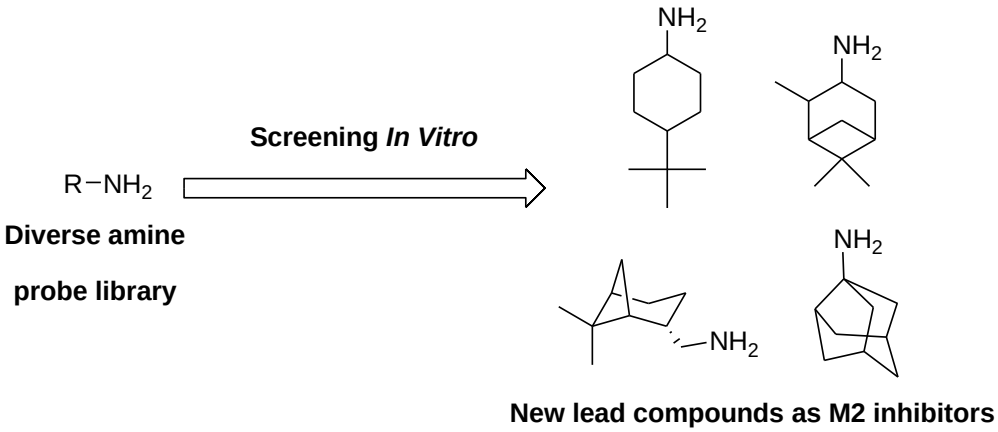

Discovery of multiple lead compounds as M2 inhibitors through the screening of a focused library of scaffold-hops

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Abstract

It is urgent to discover new anti-influenza drugs considering the threat of so called swine flu and Spanish flu. Though Adamantane derivatives are the only M2 inhibitors as anti-influenza virus A drugs, they are limited to use in the US due to drug resistant. Herein we reported that multiple lead compounds as M2 inhibitors were rapidly generated through the screening of focused library designed with scaffold-hopping strategy based on Amantadine.

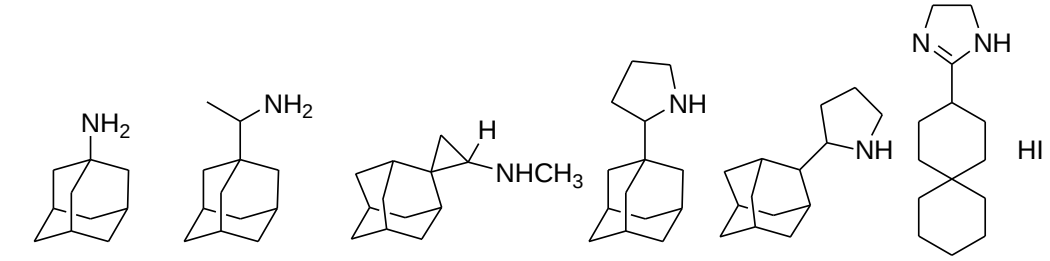
Introduction

The outbreak of new H1N1 influenza (swine flu) is spreading around the world[1,2], once again, the human faces the great threat. Although vaccination is the ideal way to prevent influenza viruses, the preparation of a new vaccine takes 6 months or more[3]. So clearly, antiviral drugs are the most important short-term resource to for human to fight the disease. However, there is no more effective drug available to combats the disease when we check our limited stocks.

The only existed ant-infuenza A drugs[3-6] are M2 inhibitor (amantadine and its derivative rimantadine) and NA inhibitors (zanamivir and oseltamivir). Even worse, amantadine and rimantadine has been limited to use for the treatment of influenza A in

the US due to the rapid development of resistance. Also, there is a growing worry that antineuraminidase-resistant viruses emerge if these drugs are widely used[7]. Considering the threat of the deadly infectious disease and very limited drugs, there is an urgent need to discover new types of M2 inhibitors for the development of new anti-influenza drugs. Though Amantadine reached the market 40 years ago, all the reported M2 inhibitors to date are adamantane derivatives (**figure 1**) with BL-1743[8] as the only one exception, this leaves many question unanswered.

Figure 1. Reported M2 inhibitors: mainly are amantadine derivatives



Amantadine **Rimantadine** **BL-1743**

For many years, high-throughput screening (HTS) of the corporation collected library did not fulfill expectations[9]. Though this strategy still plays a key role in lead generation, there is a growing interest with the design of focused library[10]. Focused screening has emerged as a more rational and focused approach that concentrates on the quality, rather than the quantity[11]. Though many publications[12-15] discussed about the trends and its application in drug discovery, there is a shortage of successful case study and most of them are in silicon.

Thus, we decided to design and screen a focused compound library (<100) of scaffold-hops[11] based on amantadine in order to generate new lead compounds in M2 inhibitor class. Herein, we not only provide a proof of concept that screening of focused library is highly efficient, but also discovered multiple lead compounds to support the anti-viral drug discovery.

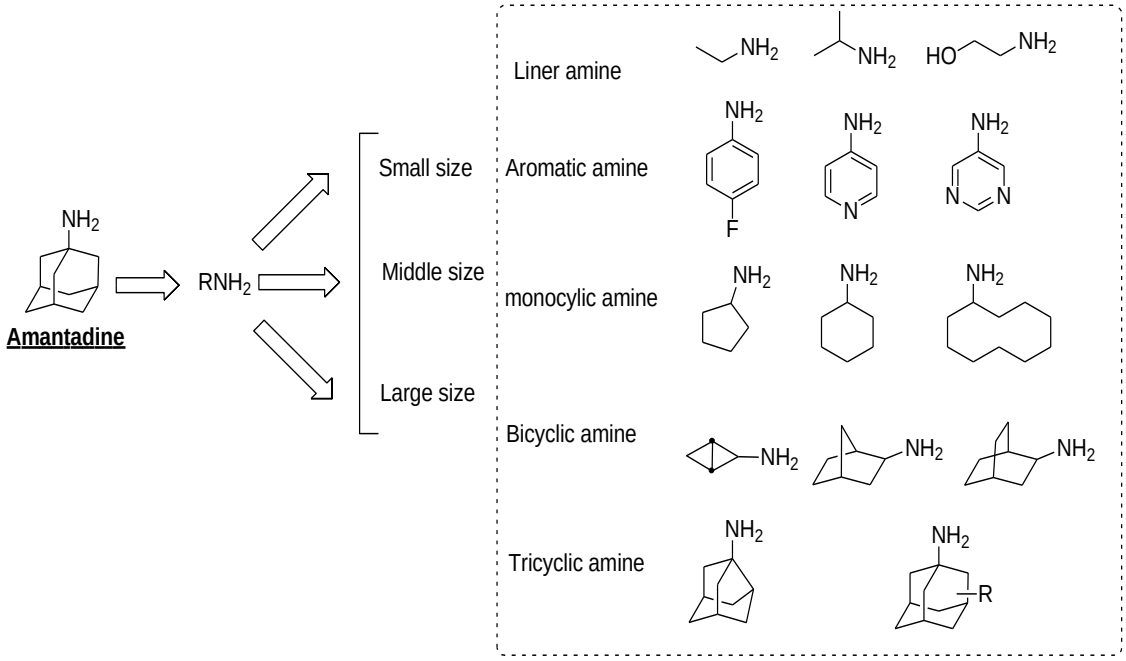
Results and Discussion

The mechanism of M2 inhibitors is to block the ion channel activity of the M2 protein of most influenza A viruses[16], and viral replication is inhibited by the blockade of hydrogen ion flow. The amino group in amantadine is probably the pharmacophore and necessary for the blockade of hydrogen ion, consequently adamantyl group is the chemotype. For unknown reason, nearly all the studies except for BL-1743 focused on

the searching of new aminoadamantane derivatives[17], less investigation was conduct on the scaffold.

The strategy of our focused library design is simply based on the structure and activity relationships of amantadine to keep the primary amine constant and diversity with the scalffold. The scaffold covers different molecular properties with emphesis in steric effect. As shown in figure 2, this library contains liner amine, aromatic amine, monocylic, bicyclic and tricyclic amines supplied by the major chemical companies. We rapidly collected the primary amine library from the commercially sources.

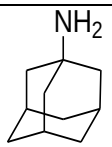
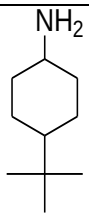
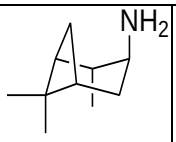
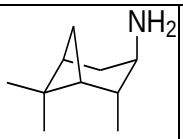
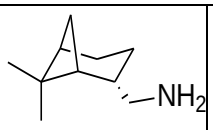
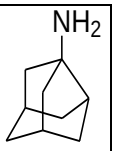
Figure 2. The design strategy of our focused library



The library was soon assayed in vitro including viral inhibition, cell based assay and patch clamp. Among of these 95 compounds, 5 compounds were found for the first time to be M2 inhibitors and are as active as Amantadine in vitro.

Table 1. Compounds as active as Amantadine in the focused library
（Chufang and Zhiyuan）

No.	Amantadine	ZSG-2-101B	LSR-2-007C	LSR-2-007D	ZSG-2-046C	ZSG-2-101E

structure						
Viral inhibition	7.447	2.438	14.37	1.323	17.88	2.029
Cell based assay	3.525	15.1	22.98	59.60	25.47	
Patch-clamp recording	8.8±2.7	4.8±1.2	6.8±2.2	4.3±2.7	4.4±1.3	13.5±4.1

IC50 (mean ± SEM) μM.

The activity data shows that the M2 ion channel can accommodate some range of chemical space, but requires minimum steric functional group to block the channel. Both liner, monocylic and aromatic amines have no effect on it, however, substituted cyclohexylmaine, some bicylcic and tricylic amine can exert inhibition closely to Amantadine. Expanding the size of Amantadine by adding substitute on the ring, such as methyl, hydroxyl does loss the activity.

Although these scalffolds along with Amantadine are not typically selected by medicinal chemists for drug development, risk-versus-benefit equation give some chances that these unfavorable scalffolds. These chemotype can be used for drug discovery against acute and deadly infectious disease. Thus, this study only provide the proof of concept that focused library is in deed practical for lead generation, but also disclosed several new M2 inhibitors for the scientists to discover new anti-influenza drugs. Drug discovery in the academia may have a good output by efficiently use screening of focused library. "chance favors the prepared mind".

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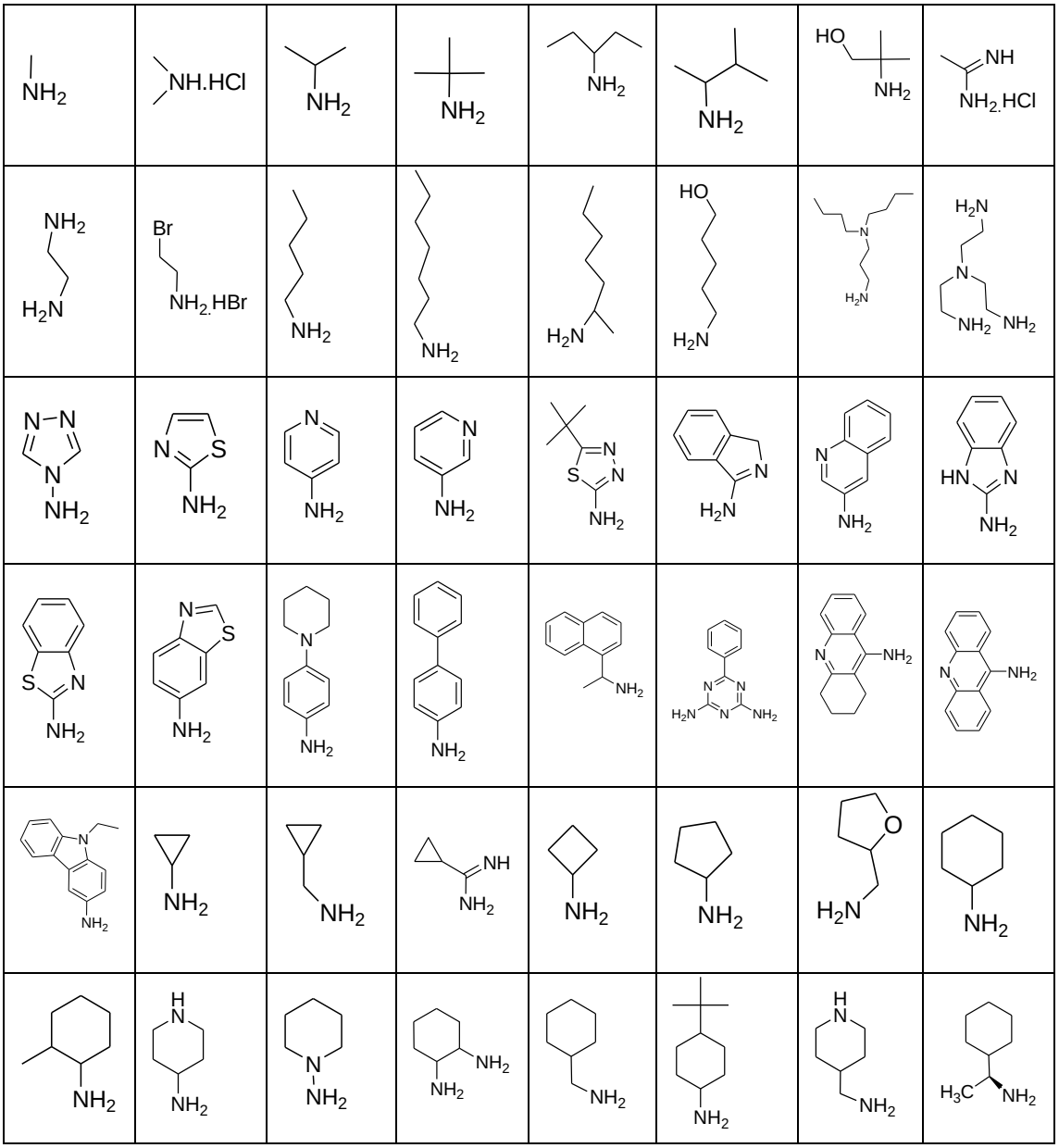
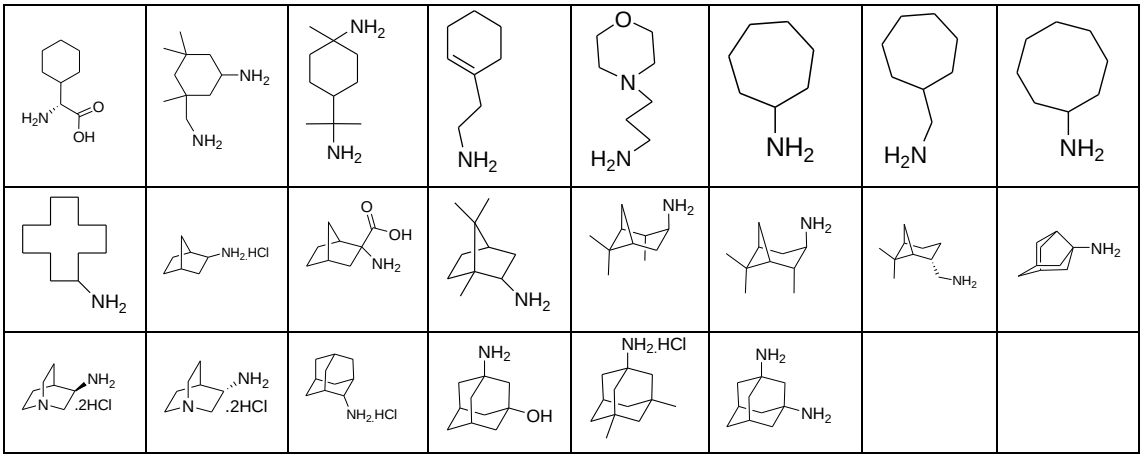
Supplementary Information

Experimental methods

Construction of focused library

All the compounds are commercially available from Aldrich, across, etc..





Viral inhibition assay (Chufang)
Protocol and data

Cell based assay(Chufang)
Protocol and data

Electrophysiological recordings by patch clamp(Zhiyuan)

M2-transformed HEK293T cells were used 24-48 hours after induction with 1 μg/ml tetracycline. Perforated whole-cell voltage-clamp recordings were carried out at room temperature (23-25°C) using an Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA) as described previously (Li et al., 2004). Recording electrodes were pulled from 1.5 mM borosilicate pipettes (World Precision Instruments, Inc., Sarasota, FL) using a horizontal puller (Model P-87; Sutter Instrument Company, Novato, CA). In the majority of experiments the extracellular solution used consisted of 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES, adjusted to pH 6.8 or 10 mM MES, adjusted to pH 5.5 and alternative pH values by the addition of either NaOH or HCl. The patch electrode had a resistance between 1.8 and 2.5 M. The pipette tip was initially filled with amphotericin-free pipette solution, containing of 130 mM Cs-methanesulfonate, 24 mM CsCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 200 μg/ml amphotericin B. The pH of the intracellular solution was adjusted to 6.8 with CsOH. Salts and drugs were obtained from Sigma-Aldrich (St. louis, MO) unless noted otherwise. The currents were filtered at 10 kHz with 16-bit accuracy using Macintosh G4 computers (Apple Computer, Cupertino, CA), ITC-16 analog-to-digital boards (Instrutech, port Washington, NY), and external operations compiled in IGOR Pro (Wavemetrics, Lake Oswego, OR). Drug applications and changes in extracellular pH were performed using a commercially available automated fast solution exchange system (RSC-200 Rapid solution changer). All data are reported as mean ± SEM for *n* number of cells. Differences in antagonist inhibition were determined from statistical

tests using IC_{50} value and a comparison between two groups was made using Student's *t* test.

Data

Cover letter:

A brief statement explaining how the manuscript meets the criteria of urgency and significance should be included in the author's cover letter.

Primary Research Formats

A Brief Communication reports a concise study of high quality, broad interest and immediate importance. This format may not exceed 3 printed journal pages. Brief Communications begin with a brief unreferenced abstract (3 sentences, no more than 70 words). The main text is typically 1,200–1,600 words (not including abstract, figure legends or references) and contains no headings. Brief Communications normally have no more than 2 display items (schemes, figures and/or tables), although this may be flexible at the discretion of the editor, provided the page limit is observed. References are limited to 25.